Malignant brain tumours are one of most critical and challenging tumors for treatment. Chemotherapy is a highly essential component of treatment of brain tumour. However there are only few chemotherapeutics available for use. This is because most of the ideal chemotherapeutics cannot attain therapeutic level in brain. This is mostly because either they do not cross BBB or are metabolized or effluxed out of brain. Available direct approaches including BBB disruption, intracerebral delivery and nasal delivery cannot be used for routine delivery because of issues including compliance and toxicity. Indirect approaches including colloidal delivery, receptor mediated transport and enhanced lipophilicity has not yet been successful in optimising delivery of chemotherapeutics.

Methotrexate is a folic acid analogue and drug of choice for treatment of many form of cancer. However it does not cross BBB in normal therapeutic doses and cannot be used in higher doses owing to high toxicity. Thus developing approaches for enhancing brain availability of MTX is essential to enable its use in brain cancer. Previously attempts have been made with this objective using various techniques including use of cetuximab (IMC-C225) dendrimer bioconjugates, osmotic blood–brain barrier disruption, intracarotid administration of short-chain alkylglycerols and transnasal delivery. However none of these have led to successful and practical use. In this scenario it is necessary to evaluate alternative approaches.

Reengineering of drugs based on the knowledge of the endogenous amino acid /oligopeptide transportation system within the BBB for brain transport is a novel approach. Amino acids are required for normal brain functioning. Being polar they cannot cross BBB passively and hence are transported across BBB via various transporters present in BBB including $ATB_{0,+}$, PEPT1, LAT1, LAT2. These transporters have high capacity and demonstrate enough substrate flexibility for transporting relatively larger molecules. Considering this several reversible conjugates of MTX were developed and evaluated for brain delivery of MTX. Both amide and ester linkage was used for reversible conjugation of amino acids with MTX.

L-glutamate is an anionic amino acid transported to brain by sodium independent x^- transporter system. This transporter has low capacity but glutamine, the amide of glutamic acid is transported by LAT1 transporter which has high substrate capacity as well as higher substrate flexibility. So MTX-GLU was developed keeping in view the structural features of glutamine. The conjugate was found to be stable enough for optimum peripheral stability. It

was also shown to hydrolyze slowly in brain homogenate suggesting slow release of MTX which is desirable for chemotherapy of cancer. Following intravenous administration the relative uptake efficiency was 5.76 with a concentration efficiency of 5.28. This suggested significant brain availability of MTX from the conjugate without any impact on toxicity profile of MTX.

Cationic amino acid L-lysine cannot be synthesized in brain and hence taken up from circulation for optimum brain function facilitated by transporter system y^+ and LAT1 at BBB. Based on this a novel lysine conjugate of MTX (MTX-LYS) was developed. It was stable enough in the plasma to survive for brain transportation. The enzymatic release of MTX from MTX-LYS was slower both in vivo and in vitro, which can be an essential feature for sustained drug action. The peripheral tissue distribution of MTX-LYS was less but brain transport was more than seven times that of MTX administered alone.

L-Phenylalanine, L-Leucine and L-Tyrosine are neutral amino acids and are transported from plasma into the brain by LAT1 at BBB. These amino acids show higher affinity for this system expressed at BBB than those expressed peripherally. Capitalizing on this L-Tyrosine conjugate of MTX (MTX-TYR) was developed. Being an ester conjugate stability was a concern. However its stability study suggested its suitability for i.v administration and circulation for brain transportation. The peripheral tissue distribution of MTX from MTX-TYR was less but brain transport was more than eight times compared to MTX administered alone. Toxic profile of the conjugate was similar to that of MTX. However upon repeated administration it showed some toxicity which is usually associated with chemotherapy. This was also because of increase in availability of MTX in brain. In continuation of this approach L-Phenyl alanine (MTX-PAL) and L-leucine (MTX-LEU) conjugate of MTX were developed. Both conjugates were found to have good permeability. However the relative uptake efficiency of MTX-LEU was higher than that of the MTX-PAL. Both conjugates were shown to release MTX slowly in brain with good peripheral stability. There was no significant effect on toxicity as compared to MTX. Peripheral tissue distributions of conjugates were found to be less compared to brain distribution suggesting some selectivity in MTX brain delivery.

This work gives an alternative approach to enhance brain delivery of MTX. Although exact mode of transport could not be established, there is enough proof of the higher brain

availability of MTX. These transporters are also expressed in tumors. So these conjugates can be used to enhance MTX uptake by tumor. However this needs further investigation.

KEYWORDS: Brain Delivery, Methotrexate, Amino acid, Conjugate, Amino acid transporters